PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 April 1997 (07.04.97)	
International application No. PCT/NL96/00317	**Applicant's or agent's file reference** PCT 0493
International filing date (day/month/year) 05 August 1996 (05.08.96)	**Priority date** (day/month/year) 03 August 1995 (03.08.95)
Applicant GEUZE, Johannes, J. et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   03 March 1997 (03.03.97)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Céline Faust
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 730.91.11

Copy for the Elected Office (EO/US) PCT/NL96/00317

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

SMULDERS, Th., A., H., J.
Vereenigde Octrooibureaux
Nieuwe Parklaan 97
NL-2587 BN The Hague
PAYS-BAS

Date of mailing (day/month/year)
22 January 1998 (22.01.98)

Applicant's or agent's file reference
PCT 0493

IMPORTANT NOTIFICATION

International application No.
PCT/NL96/00317

International filing date (day/month/year)
05 August 1996 (05.08.96)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
RIJKSUNIVERSITEIT UTRECHT
Universiteitsweg 100
NL-3584 CG Utrecht
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
UNIVERSITEIT UTRECHT
Universiteitsweg 100
NL-3584 CG Utrecht
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Nicola Wolff

Telephone No.: (41-22) 338.83.38

TENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PCT 0493		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 96/00317	05/08/1996	03/08/1995
Applicant		
RIJKSUNIVERSITEIT TE LEIDEN et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing.
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 96/00317

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claim 10 (partially when the method is carried out in vivo) is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/NL 96/00317

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 315, 1985, LONDON GB, pages 327-329, XP002016307 WALDEN P. ET AL: "Induction of regulatory T-lymphocyte responses by liposomes carrying major histocompatibility complex molecules and forein antigen" see the whole document ---	1-8,10
X	JOURNAL OF IMMUNOLOGY, vol. 151, no. 8, 1993, BALTIMORE US, pages 3988-3998, XP002016308 HARDING C.V. ET AL: "Immunogenic peptides bind to class II MHC molecules in an early lysosomal compartment" see the whole document ---	1-10
	-/--	

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 1996	05. 11. 96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Fernandez y Branas,F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 369, 1994, LONDON GB, pages 113-120, XP002016309 AMIGORENA S. ET AL: "Transient accumulation of new class II MHC molecules in a novel endocytic compartment in B lymphocytes" cited in the application see the whole document -----	1-4,6

1

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCT 0493		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL 96/ 00317	05/08/1996	03/08/1995
International Patent Classification (IPC) or national classification and IPC		
A61K39/385		
Applicant		
RIJKSUNIVERSITEIT TE LEIDEN et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 1 sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/03/1997	19. 11. 97

Name and mailing address of the IPEA

European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Netherlands
Tel.: (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Fernández y Branas, F.J.

Telephone No. 01949

I. Basis of the report

1. This report has been drawn up on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*

☐ the international application as originally filed

☒	the description,	pages	1-15	, as originally filed
		pages		, filed with the demand
		pages		, filed with the letter of
☒	the claims,	Nos.		, as originally filed
		Nos.		, as amended under Article 19
		Nos.		, filed with the demand
		Nos.	1-10	, filed with the letter of 28-08-97
☒	the drawings,	sheets / fig.	1/7-7/7	, as originally filed
		sheets / fig.		, filed with the demand
		sheets / fig.		, filed with the letter of

2. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.

☐ the drawings, sheets / fig.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2 (c)).

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10

because:

☒ the said international application, or the said claims relate to the following Nos. 10 subject matter which does not require an international preliminary examination *(specify)*:

Claim 10 relate to a method of treatment of the human or animal body by therapy in the sense of Article 34(4)(a)(i) and Rule 67.1(iv) PCT.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims are so unclear that no meaningful opinion could be formed *(specify)*: Nos.

☐ the claims, or said claims are so inadequately supported by the description no meaningful opinion could be formed. Nos.

☐ no international search report has been established for said claims Nos.

V. Reasoned statement und r Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Claims	5 7-8	YES
	Claims	1-4 6 9	NO
Inventive Step	Claims		YES
	Claims	1-9	NO
Industrial Applicability	Claims	1-4, 5 (see below), 6-7, 8 (see below), 9	YES
	Claims		NO

2. Citations and Explanations

D1.....The Journal of Immunology, Vol 151, No.8, 1993, pages 3988-3998

D2.....Nature, Vol 315, 1985, pag 27-329

1)D2 discloses liposomes having inserted class II major histocompatibility complex (MHC) molecules and protein antigens. The liposomes are shown to stimulate cloned helper T cells and T-cell hybridomas in an antigen-specific, MHC-restricted manner in the absence of antigen presenting cells (APC).

2)D1 discloses the isolation of lysosomes charged with MHC-II molecules and processed antigens. The lysosomes are isolated by centrifugation in Percoll, see page 3989, right column, second paragraph. When macrophages were exposed to hen egg lysozyme (HEL) and the lysosomes isolated, the lysosomes were able to stimulate T-cell hybridomas specific for HEL, see page 3992, left column, second paragraph.

3) Claims for products defined in terms of a process of manufacture are admissible only if the products as such fulfil the requirements for patentability, i.e. inter alia that they are new and

inventive. A product is not rendered novel merely by the fact that it is produced by means of a new process.

3.1.- In the case of claims 1-6 the product in itself is defined as being an "antigen presenting vesicle free from its natural surroundings" (claim1), "comprising at least a biological active part of a major histocompatibility complex class I or class II or a derivative thereof" (claim 2), and "which additionally comprises at least partly processed antigens" (claim 3). These are therefore the technical features defining the products of claims 1-6, regardless of the way they have been produced. This is also the interpretation made in the description of the present application, see page 6 lines 18-32.

3.2.- The expression "free from its natural surroundings" is vague and open to interpretation and therefore unclear. This expression does not provide any further positive characterisation of the vesicles claimed, it only characterizes the vesicles in a negative manner, by stating that the vesicles should not contain any of its "natural surroundings", whatever this might be (see below 3.5)

3.3.- The Applicant is however of the opinion that this expression is clear and often used to distinguish between products found in nature and its man-made isolated forms, such as isolated proteins. In this sense, both in D1 or D2 the vesicles are not free from their natural surroundings, they have only been brought into a subfraction of their natural surroundings.

3.4.-The IPEA does not agree with this interpretation. If the expression "free from its natural surroundings" is understood as "isolated", then the vesicles of D1 or D2 are unquestionably isolated too.

3.5.- Moreover, the expression "natural surroundings" may be understood in various ways (e.g. any isolated subfraction of the natural surroundings may also be understood as falling within the definition of "free from its natural surroundings"). Additionally, the constitution of this "natural surroundings" is not limited to the surroundings found in nature, but it includes also surroundings of artificial or man-made products. As said expression is interpretable i.e. it may be understood in different ways depending on the reader, it is unclear.

3.6- The expression "at least partly processed antigens" (claim 3) is vague, open to interpretation and thus unclear.

4)Thus, in view of D2, and bearing in mind point 3) above, the subject matter of claims 1-4 and 6 lacks novelty in the sense of Article 33(2) PCT.

5)Thus, in view of D1, and bearing in mind point 3) above, the subject matter of claims 1-4, 6 and 9 lacks novelty in the sense of Article 33(2) PCT.

6)Both the vesicles of D1 or the liposomes of D2 are able to stimulate T-cells in vitro in the absence of APC. For the skilled person this is a clear indication that these vesicles could be used in vaccination for the stimulation in vivo of the cellular immune response. Therefore in view of D1 or D2 the subject matter of claims 5 and 7-8 does nor involve an inventive step in the sense of Article 33(3) PCT.

7)For the assessment of the presently worded claims 5 and 8 on the question whether their subject matter is industrially applicable, no unified criteria exist in the PCT. The patentability under national patent laws can also be dependent on the formulation of the claims. The EPO, for example, does not recognise the subject matter of claims to the use of a compound in medical treatment as being industrially applicable, but will allow, however, claims to a known compound for first medical use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VIII. Certain observations on th international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The following objections concerning the requirements of Article 6 PCT arise:

The expressions "free from its natural surroundings" (claim1) and "partly processed antigens" (claim 3) are unclear.

The expression "wherein processed antigen is present in the context of major histocompatibility complex 1 or 2" (claim 4) is unclear.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 96/00317

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 315, 1985, LONDON GB, pages 327-329, XP002016307 WALDEN P. ET AL: "Induction of regulatory T-lymphocyte responses by liposomes carrying major histocompatibility complex molecules and forein antigen" see the whole document ---	1-8,10
X	JOURNAL OF IMMUNOLOGY, vol. 151, no. 8, 1993, BALTIMORE US, pages 3988-3998, XP002016308 HARDING C.V. ET AL: "Immunogenic peptides bind to class II MHC molecules in an early lysosomal compartment" see the whole document ---	1-10

-/--

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 1996	05. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Fernandez y Branas, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/NL 96/00317

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 369, 1994, LONDON GB, pages 113-120, XP002016309 AMIGORENA S. ET AL: "Transient accumulation of new class II MHC molecules in a novel endocytic compartment in B lymphocytes" cited in the application see the whole document -----	1-4,6

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claim 10 (partially when the method is carried out in vivo) is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classificati n 6 : A61K 39/385

A1

(11) International Publication Number: WO 97/05900

(43) International Publication Date: 20 February 1997 (20.02.97)

(21) International Application Number: PCT/NL96/00317

(22) International Filing Date: 5 August 1996 (05.08.96)

(30) Priority Data:
95202123.6 3 August 1995 (03.08.95) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicants *(for all designated States except US)*: RIJKSUNIVERSITEIT TE LEIDEN [NL/NL]; Stationsweg 46, NL-2312 AV Leiden (NL). RIJKSUNIVERSITEIT UTRECHT [NL/NL]; Universiteitsweg 100, NL-3584 CG Utrecht (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GEUZE, Johannes, J. [NL/NL]; Rijksuniversiteit Utrecht, Universiteitsweg 100, NL-3584 CG Utrecht (NL). MELIEF, Cornelis, J., M. [NL/NL]; Rijksuniversiteit te Leiden, Stationsweg 46, NL-2312 AV Leiden (NL).

(74) Agent: SMULDERS, Th., A., H., J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: CELL DERIVED ANTIGEN PRESENTING VESICLES

(57) Abstract

The invention provides a novel vehicle for vaccination, in particular peptide vaccination. The new vehicle has been termed an exosome. Exosomes are vesicles derived from MHC class II enriched compartments in antigen presenting cells. The exosomes possess MHC II and/or MHC I molecules at their surface and possibly peptides derived from processed antigens in said MHC's. Thus the exosome is a perfect vaccination vehicle in that it presents the peptide in a natural setting. The peptides present in the exosome in the MHC molecule may be processed by the antigen presenting cell from which the exosome is derived. Empty MHC molecules on exosomes may also be loaded with peptides afterwards.

Title: Cell derived antigen presenting vesicles.

The invention relates to the field of immunology, especially the cellular responses of the immune system, more in particular to the induction of said responses by peptides presented in the context of major histocompatibility complexes I and/or II.

It is known that antigen presenting cells take up antigens through endocytosis, whereafter these antigens are cleaved into peptides which are presented at the surface of said antigen presenting cells in the context of a major histocompatibility complex. By this presentation on the surface the peptides derived from the original antigen can be recognized by for instance helper T-lymphocytes, further activating the cellular immune response.

Thus Helper T-lymphocytes recognize exogenous antigens bound to major histocompatibility complex (MHC) class II molecules expressed by a variety of antigen presenting cells (APCs) such as B-lymphocytes, macrophages and dendritic cells (1). Compelling evidence indicates that newly synthesized α and β subunits of MHC class II in association with the invariant chain (I-chain) are transported to intracellular compartments before reaching the plasma membrane (2,3). In these compartments the l-chain is degraded and MHC class II are potentially free to bind antigenic peptides arising from the degradation of antigens internalized by the APC (1, 4). We and others have shown that most of the intracellular MHC class II molecules reside in a Iysosome-like, MHC-class II-enriched compartment (MIIC) which contains characteristic membrane vesicles and concentrically arranged membrane sheets (5, 6, 7, 8, 9, 10) . MIICs and the related CIIVs (11), likely represent the meeting point between MHC class II and antigenic peptides (8,12). Once loaded with peptide, MHC class II molecules are transferred to the cell surface via an unknown pathway for presentation to T-lymphocytes.

Electron microscopy of immunogold labeled ultra thin cryosections from several human B-lymphoblastoid cell lines revealed MIICs whose surrounding membrane was contiguous with the plasma membrane in an exocytotic fashion and showed extracellular vesicles reminiscent of those present in non-fused MIICs (Figure 1A and B). Similar secretion of vesicles, termed exosomes, has been described for reticulocytes (13). Exosomes from B cells immunolabeled for the Iysosomal membrane proteins LAMP1 (Figure 1 B) and CD63 (not shown) known to be expressed in MIICs (5, 6). Both LAMP1 and CD63 were absent from the rest of the plasma membrane. Scarce labeling for MHC class II was associated with the limiting membrane of the fused MIICs but MHC class II was enriched in the externalized exosomes (Figure 1A and B). To test the release of MIIC contents further, B cells were allowed to internalize 5 nm gold particles conjugated to Bovine Serum Albumin (BSAG), and were then washed and reincubated in the absence of BSAG. Exosomes associated with previously endocytosed BSAG began to appear in exocytotic profiles after 30 min of uptake (10 min pulse and 20 min chase) (Figure 1B) and were abundant after 50 min (10 min pulse and 40 min chase) (Figure 1A). We conclude that multivesicular MIICs of human B-cell lines can fuse with the plasma membrane thereby releasing MHC class II-rich exosomes into the extracellular milieu.

For a further characterization, exosomes were isolated from the culture media of the human B cell line RN by differential centrifugation (Figure 2). Pelleted membranes were analyzed by DS-PAGE and Western blotting. After removal of cells, the majority of MHC class II-containing membranes sediment at 70.000 g (Figure 2 A, lane 6). The 70.000 g pellets were composed of a homogeneous population of vesicles labeled for MHC class II (Figure 2 B). The vesicles were morphologically similar to those present in MIICs and in exocytotic profiles of sectioned cells (Figures 1 A and B): their size ranged from 60 to 80 nm. To obtain biochemical evidence that the secreted MHC class II is membrane bound, 70.000 g pellets were fractionated by floatation in linear

sucrose gradients (14). Western blot analysis of the non-boiled and non-reduced gradient fractions showed that MHC class II molecules floated to an equilibrium density of 1.13 g/ml, confirming their association with membrane vesicles (Fig. A). MHC class II molecules recovered from the gradient fractions were predominantly in the SDS-stable, compact form indicating their stabilization by bound peptides (15). Together, these results show that the secreted MHC class II is associated with membrane vesicles and has bound peptides. To determine the kinetics and the extent to which newly synthesized MHC class II molecules are released into the medium, RN cells were metabolically pulse-labeled for 45 min. with [35S]-methionine and chased for up to 24 hours in the absence of label (16). After pulse-labeling MHC class II immunoprecipitated as SDS-unstable α-β-1-chains complexes (Fig.3 B, lane 0). At 6 hours of chase part of MHC class II molecules were converted to SDS-stable, α-β-peptide complexes consistent with the kinetics reported for other human B cell lines (2, 17). Recovery of [35S]-compact MHC class II from pelleted exosomes started at 12 hours and amounted 10 + 4% (n=5) of the total newly synthesized MHC class II after 24 hours of chase. The relatively slow rate by which newly synthesized MHC class II was secreted into the medium suggests that insertion from the limiting membrane of MIICs into the plasma membrane during exocytosis is probably not the only pathway by which MHC class II molecules are delivered to the cell surface. To test the possibility that the vesicles recovered from the medium represented shed plasma membrane fragments or cell debris instead of exosomes, cells and exosome preparations were biotinilated and the patterns of the biotinilated proteins were studied by Western blotting with 125I-Streptavidin (18). Figure 3 C reveals differential patterns of biotinilated proteins in exosomes and plasma membranes. Whereas plasma membranes show a broad spectrum of biotinilated proteins (Figure 3C, lane 2), two proteins are enriched in exosomes (Figure 3C, lanes 3 and 4). Immunoprecipitation of the biotinilated exosomal proteins with

a monoclonal anti-class II antibody (19) identified these proteins as MHC class II (α and β subunits (Figure 3C, lane 1). Furthermore, the exosomes contain two minor bands at higher molecular weight which are not clearly detected in plasma membranes (Figure 3C, lanes 3 and 4). These proteins were also immunoprecipitated with the anti-class II antibody (Figure 3C, lane 1). To test the unlikely possibility that plasma membrane fragments eventually present in the 70.000 g pellets contributed to the enrichment of MHC class II in exosomes, biotinilated cells were homogenized and the homogenates were processed as the cell culture supernatants (18). Very low amount of membranes are pelleted at 70.000 g and these show a pattern of biotinilated proteins matching that of total plasma membrane, as expected (Figure 3C, lane 5). When the cells were metabolically labeled with [35S]-methionine for 45 min. and chased for up to 24 hours (16), the [^{35}S]-Transferrin receptor (TfR) ([^{35}S]-TfR) did not appear in exosomes at any chase time (data not shown). TfR is present at the plasma membrane of B cells but is absent from MIIC (8, 10). Together, these observations emphasize that exosomes are not derived from shed plasma membranes but represent an unique population of MHC class II- enriched membrane vesicles.

Since the luminal domain of MHC class II molecules is exposed at the outside of exosomes (20), exosomes may be able to present antigens to T cells. To test this hypothesis, isolated exosomes were allowed to bind peptide 418-427 from the model antigen HSP 65 of Mycobacterium Leprae. The exosome preparations were then added to the T cell clone 2F10 which recognizes this peptide in the context of HLADR15 (21). In a parallel experiment, RN cells were allowed to endocytose HSP65 protein continuously for 24 hrs, washed, and incubated in the absence of antigen for another 24 hrs (22). Both, exosomes incubated with antigenic peptide (Figures 4 A and C) and exosomes derived from cells that were pre-incubated with antigen (Figures 4 B and D) were able to induce a specific T cell response (23). A half maximal response was obtained with an amount of exosomes secreted by 3 x 10^5 RN cells in 24 hours

(Fig.4 D). In comparison $2x10^4$ intact RN cells were necessary to achieve the half maximal response (Fig.4 B, 24). The responses observed were DR restricted. Anti-HLA-DR antibody blocked T cell proliferation completely, whereas antiHLA-DP was ineffective (Figs 4 B and D). From these data we conclude that culture media of B cells provide for a source of MIIC-derived microvesicles (exosomes) that can induce T cell responses by themselves (25).

Exocytosis of MIIC vesicles by B-lymphocytes is reminiscent of the exocytosis of the vesicles contained in the cytolytic granules of cytotoxic T-lymphocytes (CTLs) (26). Both MIICs and cytolytic granules have lysosomal characteristics and contain internal membranes. The internal vesicles of cytolytic granules are exocytosed by the CTLs upon CTL-target cell interaction and presumably have a role in the killing of target cells (26). Whether B-cell exosomes also have an extracellular role *in vivo* remains to be established. It has been suggested that follicular dendritic cells acquire MHC class II molecules released from surrounding B cells by an unknown mechanism (27). It is worth studying the possibility that exosomes serve as carriers of MHC class II-peptide complexes between different cells of the immune system. Whether physiological APCs like dendritic cells and macrophages generate exosomes has to be studied (28). However, secretion of lysosomal contents by macrophages has been documented and macrophage tubular lysosomes are rich in MHC class II and contain membrane vesicles (29). It can be speculated that *in vivo,* exosomes may function as transport vehicles for MHC class II-peptide complexes responsible for maintenance of long term T cell memory or T cell tolerance. Finally, since exosomes can easily be obtained and are capable of presenting antigens specifically and efficiently, it is worth exploring their usefulness as biological vehicles in immunotherapy.

The invention therefore provides an antigen presenting vesicle free from its natural surroundings obtainable from

antigen presenting cells, such as B-cells, macrophages or dendritic cells, especially Langerhans cells of the epidermis.

These vesicles preferably will contain major histocompatiblility complex (MHC) I and/or II, most preferably loaded with a peptide derived from or corresponding to an antigen which can be processed by antigen presenting cells.

It has been tried before to produce similar vesicles synthetically, for instance in the form of liposomes, but these attempts have sofar not been successful. Now that we have surprisingly found that there are counterparts of said liposomes in nature, these counterparts can of course be used in any intended application of said liposomes.

The major advantage of the vesicles according to the invention is of course that they will automatically comprise all the necessary elements for antigen presentation. Further analysis of the vesicles, once discovered will therefore result in a better understanding of which elements are essential for said presentation on said vesicles. It will then of course be possible to arrive at vesicles according to the invention in other ways then by isolation from cells. The invention therefor does encompass all antigen presenting vesicles which comprise the essential elements for presenting such antigens, regardless of the way they are produced or obtained.

One may for instance think of synthetically prepared liposomes, provided with at least biologically active parts of (recombinant) MHC I or II, optionally provided with processing agents for antigens to be presented in the context of said MHC. Of course cells which produce these vesicles can also be provided with recombinant MHC I or II encoding genes, so that the desired MHC's will be present on the eventually resulting vesicles, etc.

Although vesicles which present peptides in the context of MHC I or II are preferred, it is also very useful to produce vesicles which do have the MHC's on their surface, but without a peptide being present therein. These vesicles can

then be loaded with desired peptides having the right binding motiv to fit in the respective MHC.

The first and perhaps foremost use of these vesicles that comes to mind is of course mimicking their role in nature, which is the presentation of peptides as antigens, for the stimulation of for instance T-cells. Thus the vesicles according to the invention can be very suitably used in for instance vaccines. These vaccines can be designed to elicit an immune response against any proteinaceous substance which has peptide antigens that can be presented in the context of MHC.

The vaccines may of course comprise suitable adjuvants, if necessary, carriers, if necessary, ecxipients for administration, etc.

The vaccines can be used in the treatment or prophylaxis of many disorders, such as infections, immune disorders, malignancies, etc.

Very important applications will of course be the treatment or prophylaxis of AIDS, eliciting immuneresponses agains tumours and the like.

Another important application of the vesicles according to the invention is that they may be used to induce tolerance to certain antigens, for instance by giving large doses of the vesicles orally.

Based on the description of the invention and specifically referring to the following experimental part illustrating the invention the person skilled in the art will be able to find further uses of the vesicles according to the invention without departing from the spirit of the invention.

Legends to Figures:

Figure 1:

MIICs are exocytotic compartments. T2-DR3 cells were incubated in the presence of 5 nm BSAG for 10 min., washed, chased for 40 min. and processed for cryoultramicrotomy as described (30). Ultrathin cryosections were immunolabeled with a rabbit polyclonal anti-class II antibody (5) and antibody binding sites were visualized with protein A conjugated to gold (PAG with sizes in nm indicated on the figures). MHC class II labeling is present at the limiting membrane of the exocytotic profile and on the exosomes. The profile also contains abundant re-externalized BSAG particles. PM: plasma membrane. B, RN cells were pulsed with BSAG for 10 min. and chased for 20 min. Ultrathin cryosections were double-immunolabeled with anti-class II antibody and with a monoclonal anti-LAMP1 antibody (31) as indicated. One of two neighboring profiles is shown, exocytotic profile containing BSAG and numerous exosomes labeled for MHC class II and LAMP1.Bars, 0.1 μm.

Figure 2:

Isolation of exosomes from cell culture media. A, RN cells were washed by centrifugation and re-cultured in fresh medium for 2 days. Cell culture media (35 ml) containing 2-5 $x10^8$ RN cells were centrifuged twice for 10 min. at 300 g (lane 1, first run; lane 2, second run). Lane 1 contains material from 0.6 x 10^6 cells. Membranes in the culture medium from 2-5 x 10^8 cells were pelleted by sequential centrifugation steps: twice at 1200 g (lane 3 and 4), and once at 10.000 g (lane 5), 70.000 g (lane 6) and 100.000 g (lane 7). The pellets were solubilized at 100°C under reducing conditions and analyzed by Western blotting using [^{125}I]-protein A. Per lane, samples equivalent to 1 $x10^6$ cells were loaded. MHC class II α and β chains were recovered mainly from the cells (lane 1) and from the 70.000 g pellet (lane 6). B, whole mount electron microscopy of the 70.000 g pellet

immunogold labeled for MHC class II. The 70.000 g pellet was resuspended in RPMI medium, adsorbed to Formvar-carbon coated EM grids, fixed with 0.5 % glutaraldehyde in 0.1 M phosphate buffer, immunolabeled with rabbit polyclonal anti-class II antibody and 10 nm PAG and stained using the method described for ultra-thin cryosections (30). The pellet is composed of 60-80 nm vesicles showing abundant MHC class II labeling. Bar, 0.2 μm

Figure 3:

A, MHC class II present in the media are membrane bound. Membranes pelleted from culture media at 70.000g after differential ultracentrifugation were fractionated by floatation on sucrose gradients, and the non-boiled and non-reduced fractions analyzed by SDS-PAGE and Western blotting with the rabbit polyclonal anticlass II antibody (17). MHC class II molecules were recovered in fractions 5 to 12 corresponding to densities of 1.22-1.10 g/ml. The majority of MHC class II was in the SDS-stable compact form with a MW of ~ 56-60 kD (Coc/β).

B, Release of newly synthesized MHC class II molecules. RN cells were pulse-labeled with [^{35}S] methionine for 45 min. (lane 0) followed by chases in the absence of label for 6, 12 and 24 hours. MHC class II molecules were immunoprecipitated from Iysates of the cells and pelleted exosomes with the monoclonal DA6.231 anti-class II antibody (18). Immunoprecipitated MHC class II molecules were dissociated from the sepharose beads at non-reducing conditions at room temperature and analyzed by SDS-PAGE and fluorography. After pulse-labeling (0), MHC class II immunoprecipitated from the cells as SDS-unstable complex of α-β-invariant chain. SDS-stable α-β dimers were recovered from the cells after 6 hours of chase and the signal increased thereafter. In the exosomes pellets SDS-stable αβ dimers started to appear at 12 hours. C, Exosomes and plasma membrane display different patterns of biotinilated proteins (18). In plasma membranes (lane 2) and experimentally produced remnants of plasma membranes (18) many

biotinilated proteins are detected with ^{125}IStreptavidin (lane 5). In exosomes (lanes 3 and 4, show increasing concentrations of exosomes, respectively) two major proteins with a MW of 60-70 kD are detected. Lane 1 shows the immunoprecipitation of biotinilated class II α and β chains from exosomes Iysates. In these assay the higher electrophoretical mobility of α and β chains is due to their efficient binding to biotin. Two minor bands at a MW of 200-300 kD are detected in exosomes (lanes 1, 3 and 4, arrows) and are absent from the plasma membrane.

Figure 4:

Presentation of HSP 65 antigen by HLA-DR15 positive RN B cells and exosomes to the $CD4^+$ T cell clone 2F10 (22). Proliferative responses to naive cells (A), to cells pre-incubated with antigen (B), to exosomes derived from naive cells (C) and to exosomes derived from cells pre-incubated with antigen (D). The closed symbols show proliferation measurements after addition of HSP 65 derived peptide (418-427), the open symbols where peptide was not added. HLA-class II restriction was determined by adding 10 µg/ml anti-DR antibody (triangles), anti-DP (circles), or no antibody (squares). The exosomes at the highest concentration were derived from media of 1.6 x 10^6 cells. All assays were performed in triplicate and results are expressed in cpm [^{3}H]-thymidine incorporated into T cells. The SEM for triplicate cpm measurements was less then 10%. Results shown form a representative example of experiments performed in duplo.

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14. The 70.000 g pellet obtained after differential centrifugation of the cell culture supernatants of RN B

lymphoblastoid cells was resuspended in 5 ml of 2.5 M sucrose, 20 mM Hepes/NaOH pH 7.2. A linear sucrose gradient (2 M-0.25 M sucrose, 20 mM Hepes- NaOH, pH 7.2) was layered over the exosome suspension in a SW27 tube (Beckman) and was centrifuged at 100.000 g for 15 hrs. Gradient fractions (18 x 2 ml) were collected from the bottom of the tube, diluted with 3 ml PBS and ultracentrifuged for 1 hr at 200.000 g using a SW50 rotor (Beckman). The pellets were solubilized at room temperature in SDS-sample buffer lacking -- mercaptoethanol and analyzed by SDS-PAGE and Western blotting using ^{125} l-Protein A.

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16. RN cells were pulsed for 45 min. with 50 Mbq/ml [35S]-methionine (Tran-Slabel, ICN, CA) and chased for different periods of time (5x107 cells per time point). After pulse-chase labeling, the cells were pelleted by centrifugation for 10 min. at 300 g. The supernatants were collected and centrifuged for 5 min. at 10.000 g and then for 30 min. at 200.000 g in a SW60 rotor (Beckman). Cells and the 200.000 g pellets were Iysed and MHC class II and TfR were immunoprecipitated from equal samples of the Iysates. TfR was immunoprecipitated as described previously [W. Stoorvogel, H. J. Geuze, J. M. Griffith, A. L. Schwartz, G. J. Strous, J. *CellBiol.* **108**, 2137-2148 (1989)]. MHC class II was quantitated using a Phosphoimager.

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18. RN cells (2 x 10^8) were washed 3 times with ice cold PBS and incubated for 30 min. at 0°C with 1mg/ml Sulfo-NHS-biotin (Pierce). Biotin was quenched for 30 min. with 50 mM NH4 Cl . After washing with ice cold PBS, half of the cells were solubilized in SDS-sample buffer supplemented with β-mercaptoethanol. The remaining biotinilated cells were homogenized. The homogenates were centrifuged and

ultracentrifuged identically to the cell culture supernatants and the 70.000 g pellets solubilized in SDS-sample buffer supplemented with β-mercaptoethanol (control for plasma membrane remnants). Exosome preparations (70.000 g pellets of cell culture media from 2 x 10 8 cells) were biotinilated as described above and solubilized in SDS-sample buffer supplemented with β-mercaptoethanol. MHC class II was immunoprecipitated from a sample of biotinilated exosomes with the monoclonal anti-class II antibody DA6.231 (19). The biotinilated cell membranes, biotinilated exosomes and immunoprecipitated MHC class II were analyzed by SDS-PAGE and Western blotting with ^{125}I-Streptavidin.

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20. The internal MIIC vesicles are formed by inward budding of the limiting membrane of MIICs (see figures 16 and 17 in reference [6] similar to the process described for multivesicular bodies in other cell types [B. van Deurs, P. K. Holm, L. Kayser, K. Sandvig, S. H. Hansen, *Eur. J. Cell Biol.* **61**, 208-224 (1993)].

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22. The EBV-B cell lines RN (HLA-DR 15+) and JY (HLA-DR15-) were incubated in the presence or absence of purified HSP 65 protein from Mycobacterium Leprae (50μg/ml) [J.E.R. Thole, et al., *Microbial Pathogenesis* **4**, 71-83 (1988)] for 4 hr in 10 ml serum free RPMI at 2 x 10^6 cells /ml, followed by the addition of 30 ml RPMI supplemented with 10% fetal calf serum (FCS) for 20 hr at 37°C. The cells were then washed to remove free antigen and incubated further for 24 hrs in RPMI/10% FCS medium at 37°C. Exosomes were prepared by differential centrifugation (Figure 2) and the efficiency of HSP 65 antigen presentation was measured by culturing 10.000 cells of

the T cell clone 2F10 with irradiated (6.000 rad) EBV cells. B cells or exosomes resuspended in 100 µl IMDM /10% pooled human serum were added to the T cell clone (50 µl IMDM /10% pooled human serum per well) in 96 well flatbottom microtitre plates (Costar, The Netherlands) for 4 days at 37°C, 5% C02 in humidified air. When indicated, 5 µg/ml of HLA-DR15 restricted epitope of HSP65 (peptide 418-427) was added to the exosomes. Sixteen hours before termination 0.5 µCi of [^{3}H]-thymidine was added to the wells. The cells were then harvested on glass fiber filters using an automatic cell harvester and the [^{3}H]-thymidine incorporation into cell DNA was determined by liquid scintillation counting. The results are expressed as the mean of triplicate measurements).

23. As a control, exosomes were prepared from culture media of an equivalent amount of DR15-negative JY cells that have been incubated or not with antigen. JY cells secreted an equivalent amount of exosomes but these were ineffective in stimulating T cell proliferation. From these data exosomes appear to be 16 times less efficient in antigen presentation. However, in antigen presentation assays contact between B and T cells may be more efficient due to sedimentation of cells.

24. Exosomes isolated from the culture medium of the murine B cell line TA3 (l-E^{k+}) incubated in the presence a RNase-derived peptide (aa 90-105) were also capable of stimulating IL2 secretion by WA.23 cells.

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28. A number of studies documented the presence of intact MHC class II molecules in 100.000 g fractions from B cell culture media and their association of with membrane

lipids [S. G. Emerson, R. E. Cone, J. *Immunol.* **122**, 892-899 (1979);
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PCT 0493

CLAIMS

1. Antigen presenting vesicle free from its natural surroundings obtainable from antigen presenting cells.
2. Vesicle according to claim 1, comprising at least a biologically active part of an major histocompatiblity comlex class I or class II or a derivative thereof.
3. Vesicle according to claim 2 which additionally comprises at least partly processed antigens.
4. Vesicle according to claim 3 wherein processed antigen is present in the context of major histocompatibility complex 1 or 2.
5. Vesicle according to anyone of the aforegoing claims for use as a therapeutical.
6. Vesicle according to anyone of the aforegoing claims which is derived from a B-lymphocyte, a macrophage or a dendritic cell.
7. Vaccine composition comprising a vesicle according to anyone of claims 1-4 together with a usual adjuvans or carrier.
8. Use of a vesicle according to anyone of claims 1-4 in the preparation of a medicament for the treatment or prophylaxis of immune disorders or infections.
9. Method for the preparation of a vesicle according to anyone of claims 1-4, comprising the steps of differential centrifugation of membrane fractions of cell culture supernatants or lysates and recovery of the fraction containing said vesicles.
10. Method for stimulating a T cell response comprising the step of contacting T cells with a vesicle according to claim 3 or 4.

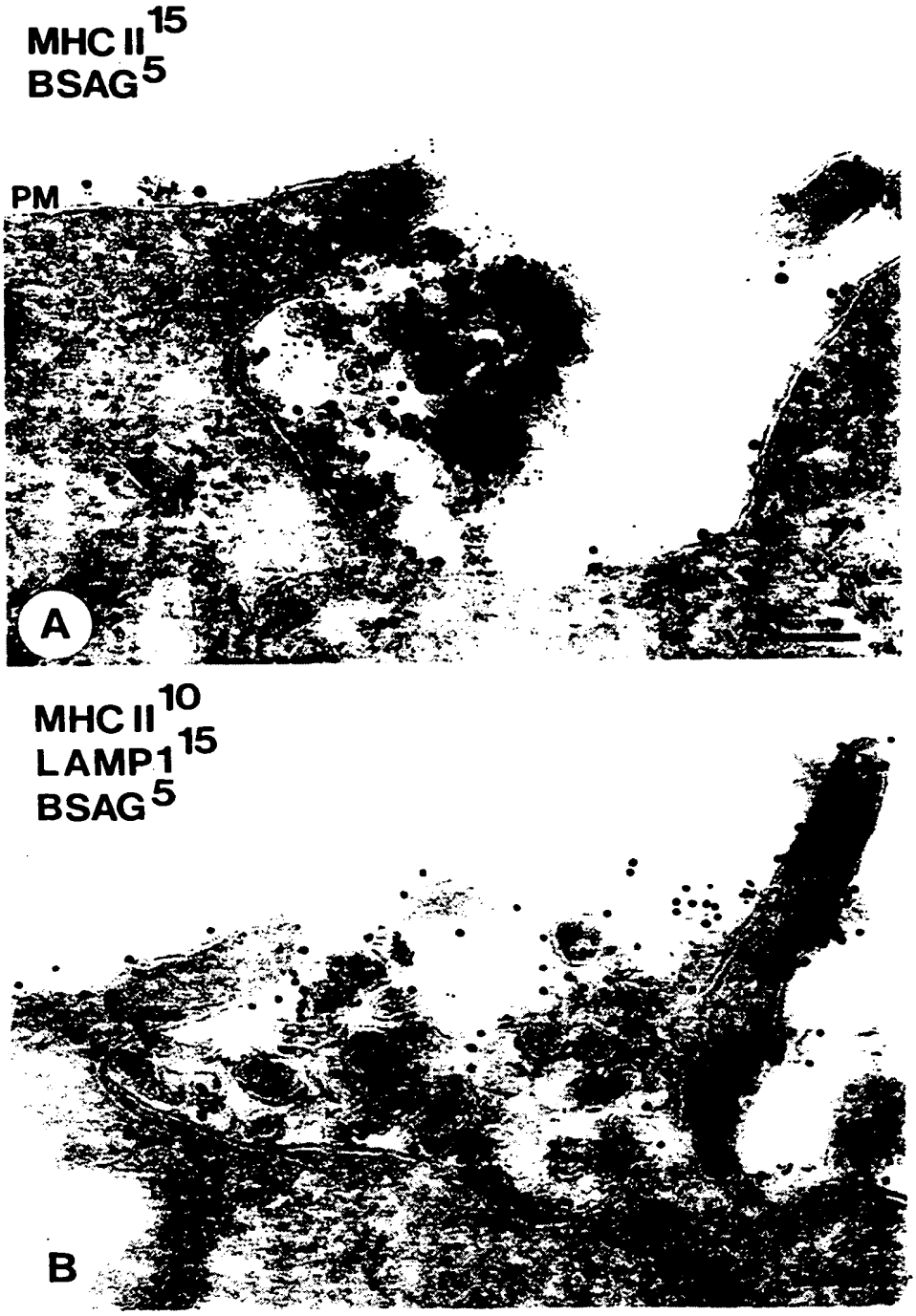


FIG. 1

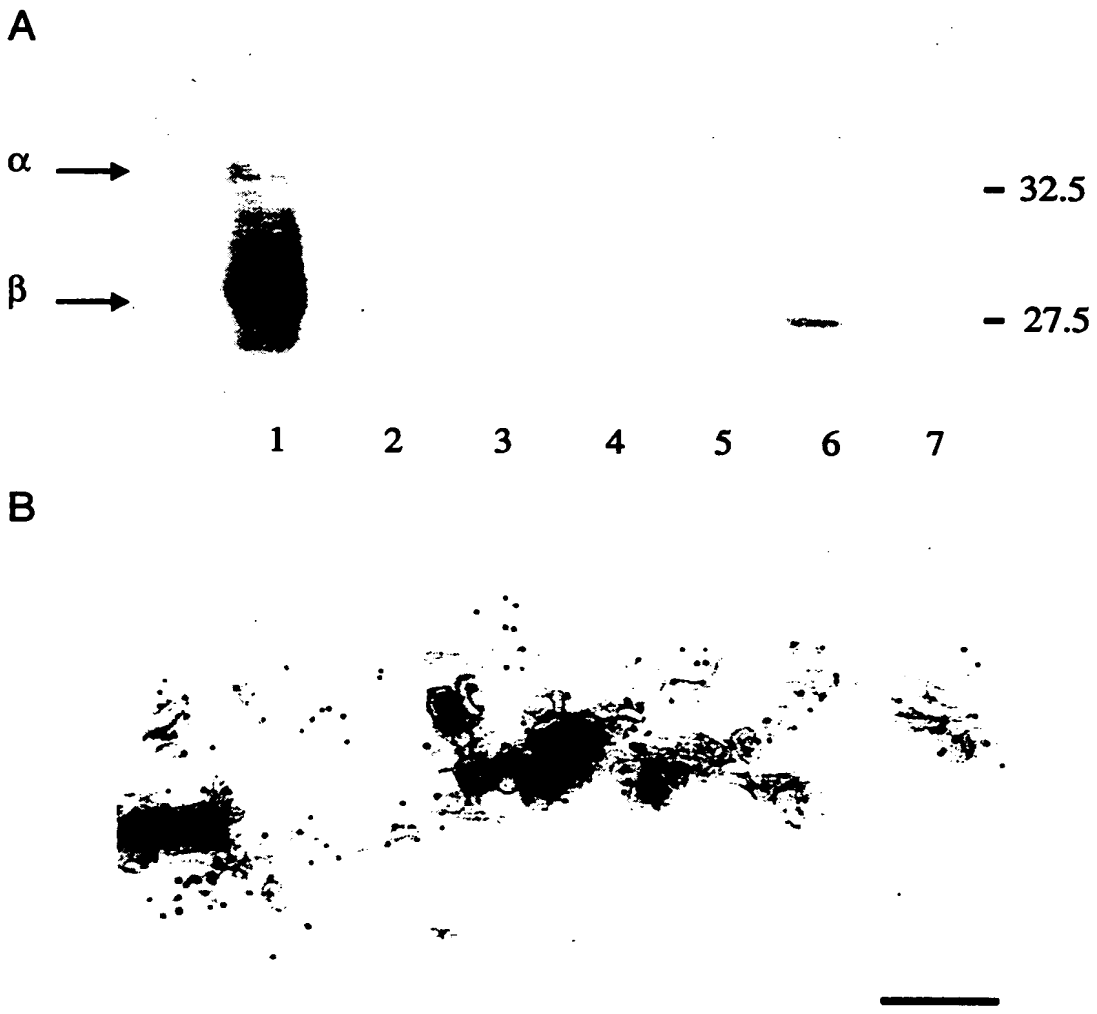


FIG. 2

A

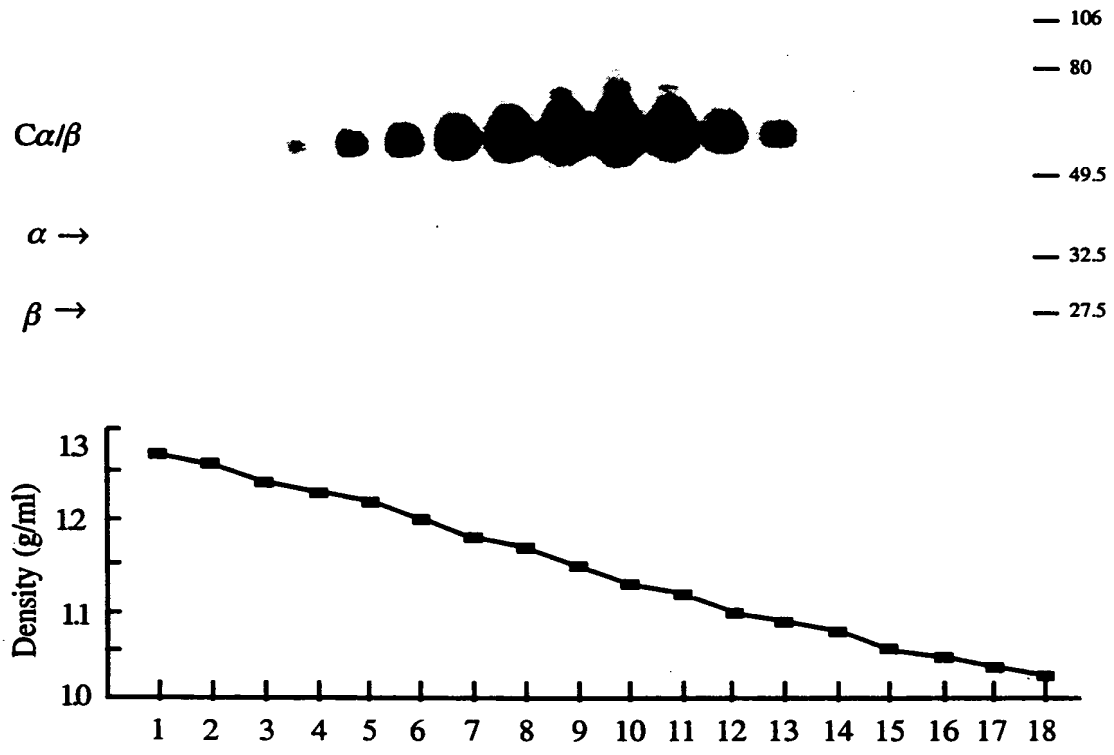

106
80
49.5
32.5
27.5
Cα/β
α →
β →
Density (g/ml)
13
12
11
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

FIG. 3

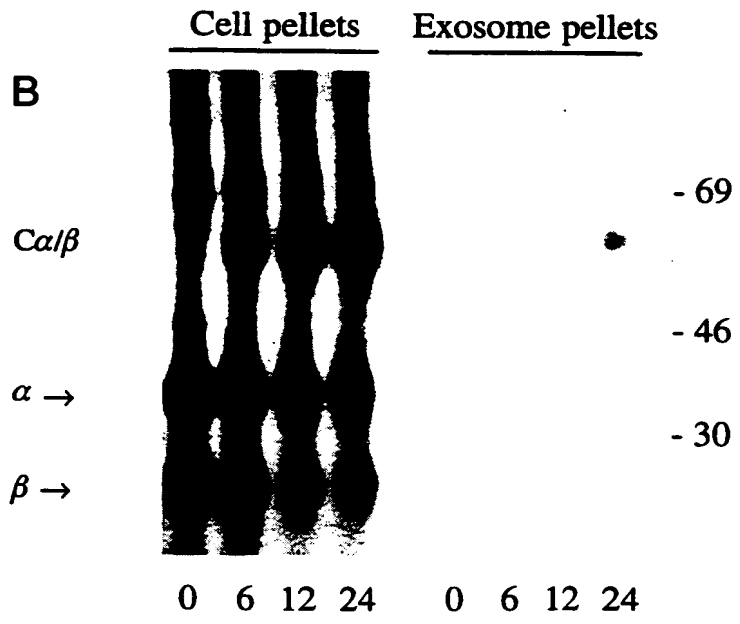


FIG. 3

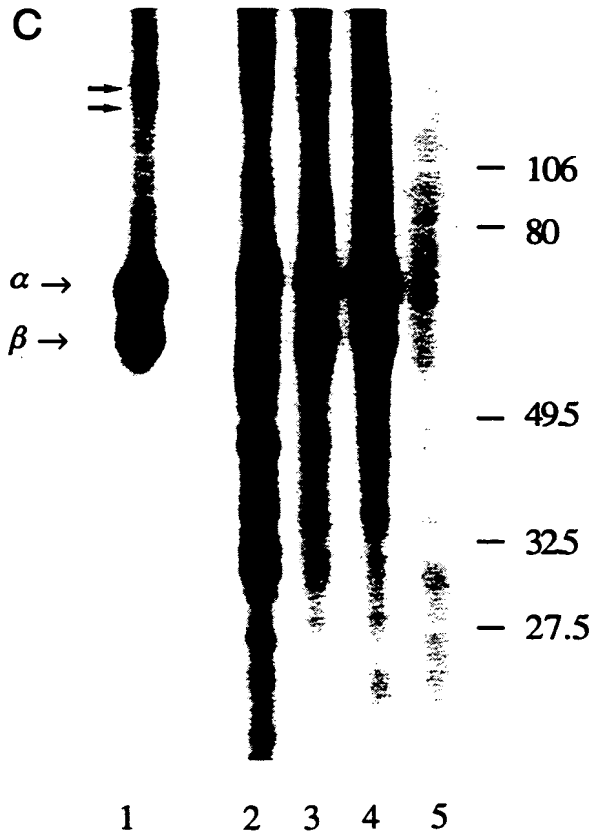


FIG. 3

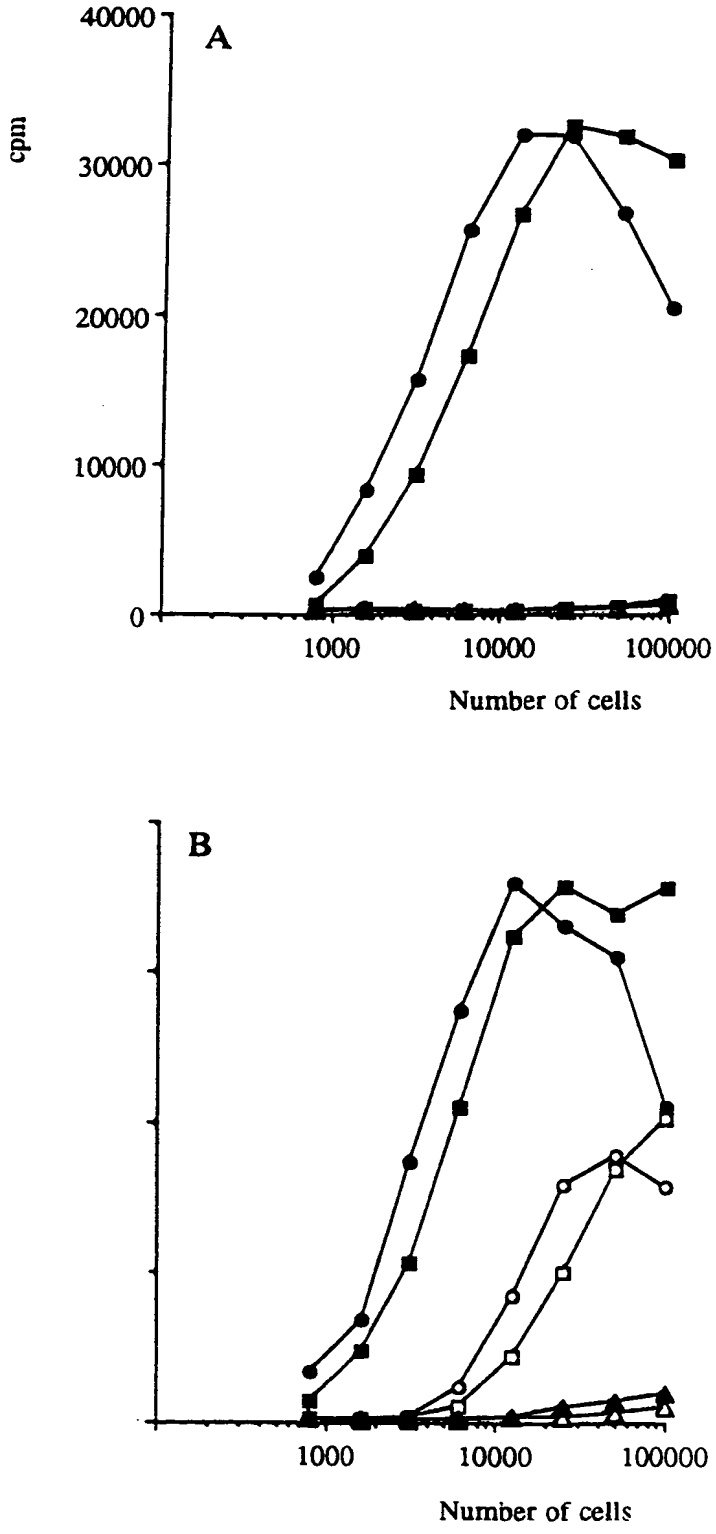
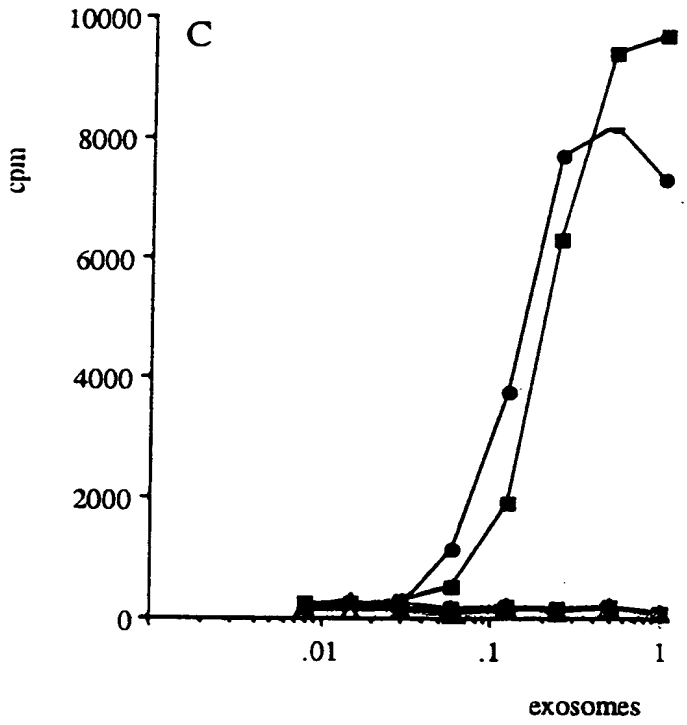

A
cpm
40000
30000
20000
10000
0
1000
10000
100000
Number of cells
B
1000
10000
100000
Number of cells

FIG. 4


C
10000
8000
6000
4000
2000
0
cpm
.01
.1
1
exosomes

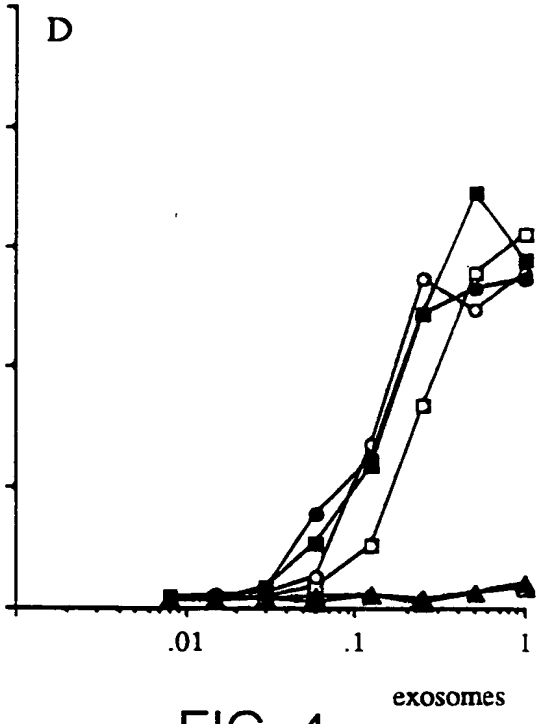

D
.01
.1
1
exosomes

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 96/00317

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 315, 1985, LONDON GB, pages 327-329, XP002016307 WALDEN P. ET AL: "Induction of regulatory T-lymphocyte responses by liposomes carrying major histocompatibility complex molecules and forein antigen" see the whole document ---	1-8,10
X	JOURNAL OF IMMUNOLOGY, vol. 151, no. 8, 1993, BALTIMORE US, pages 3988-3998, XP002016308 HARDING C.V. ET AL: "Immunogenic peptides bind to class II MHC molecules in an early lysosomal compartment" see the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 1996	05. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/NL 96/00317

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claim 10 (partially when the method is carried out in vivo) is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 369, 1994, LONDON GB, pages 113-120, XP002016309 AMIGORENA S. ET AL: "Transient accumulation of new class II MHC molecules in a novel endocytic compartment in B lymphocytes" cited in the application see the whole document -----	1-4,6